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Determination of Occupational Exposure to Depleted Uranium Based on Inductively Coupled Plasma Mass Spectrometry

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Abstract

We used a simple method based on inductively coupled plasma mass spectrometry (ICP-MS) to determine the isotopic composition of uranium in urine at levels that indicate occupational exposure to depleted uranium (DU). DU exposure is indicated by a range for ^{235}U between 0.72 and 0.2 percent. Using the ICP-MS isotopic ratio method, we determined the percentage of ^{235}U in a series of urine samples based on the measured intensities of the ^{235}U and ^{238}U ions. In this study, urine samples from 12 individuals suspected of having been exposed to DU were processed by the Armed Forces Radiobiology Research Institute (AFRRI) and then analyzed by ICP-MS at the Army Research Laboratory for the presence of DU. We achieved a lower detection limit of 14 ng L^{-1} in the original urine samples, which is at or near uranium levels in the general population (not occupationally exposed to uranium). We concluded that this approach can be used to determine the percentage of ^{235}U in urine samples characterized by higher than normal uranium levels, and thus whether occupational exposure to DU has occurred.

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1. Introduction

The Gulf War conflict was the first major combat situation involving the widespread use of depleted uranium (DU), both in armor-piercing munitions and for enhancing the armor protection and survivability of U.S. tanks. The possible exposure of military personnel to DU during this conflict renewed interest in toxicology studies involving uranium and in assays for DU [1]. In 1993, the Office of the Army Surgeon General identified a number of soldiers who had been wounded by friendly fire and possibly exposed to DU. Investigations of the health effects of DU are currently being carried out at the Armed Forces Radiobiology Research Institute (AFRRI) and the Lovelace Respiratory Research Institute (LRRI). In support of the research efforts at AFRRI, we used inductively coupled plasma mass spectrometry (ICP-MS) to determine the isotopic ratios of uranium from the urine of 12 subjects currently enrolled in the Baltimore Veterans Administration Medical Center follow-up program.

Techniques commonly used to assay uranium in environmental, geological, and biological samples include fluorescence-type techniques such as laser-induced fluorescence (LIF) [2–8] and kinetic phosphorescence analysis (KPA). Other methods that can be applied to the analysis of uranium in urine- and serum-type samples include α-spectroscopy [9–11], spectrophotometry [12], neutron activation analysis (NAA) [13], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [14], and ICP-MS [15–28]. Most of the indicated techniques require elaborate sample preparation procedures or extended data collection times, or they are subject to interference problems [27]. The LIF technique is quite useful for the determination of occupational exposure to uranium based on urine sampling, but has the drawback of an ultimate detection limit in the low nanograms per milliliter (parts per billion, ppb) range. Uranium concentrations in urine samples from the normal or unexposed population are typically in the 6 to 30 ng L^{-1} or parts per trillion (ppt) range. The NAA technique is characterized by excellent detection limits for uranium, but requires extensive sample preparation, is very labor intensive, and is not very cost effective [23]. ICP-MS and α-spectroscopy are the only techniques from this group that allow for the determination of isotopic ratios. However, in order to produce accurate results for uranium levels below 25 ppt, α-spectroscopy requires extended data collection times, while ICP-MS requires much shorter sampling times and also exhibits an instrumental detection level (IDL) of less than 1 ppt for uranium. In addition, following sample preparation, ICP-MS analyses can be accomplished in sampling times on the order of a few minutes. We therefore selected the ICP-MS isotopic ratio approach as the method of choice to assess occupational exposure to DU.

Naturally occurring uranium contains 0.72 wt.% ^{235}U, while DU typically assays for ^{235}U at 0.2 to 0.25 wt.%. A quick survey technique based on isotopic ratios would provide a useful tool for assessing whether uranium detected in bodily fluids or tissue samples was from naturally occurring sources, such as drinking water and food stocks, or from DU contamination. If the measured $^{235}U/^{238}U$ ratio indicates ^{235}U less than 0.72 wt.%, then DU exposure is verified.

We used a Perkin Elmer ELAN 6000 ICP-MS* operated in the isotopic ratio analysis mode to determine the relative concentrations of ^{235}U and ^{238}U ions in a series of urine samples. Since the obtained analytical results are expressed as the ratio of the measured intensity of the ^{235}U ion to the measured intensity of the ^{238}U ion, no standardization was required. The precision of this approach can range from a few percent to as low as 0.1 percent, depending on the ratio of the isotopes to be determined and the total ion counts obtained during the measurement. The ELAN 6000 ICP-MS uses an argon plasma as an ionization source and a quadrupole to mass analyze the ions produced. It can provide quantitation for about 80 percent of the elements in the periodic table, with typical detection limits below 1 ppt or 1 ng L^{-1}. The dynamic range is over six orders of magnitude.

In this work, we used a quick-turnaround method based on ICP-MS to measure the isotopic ratio of uranium from urine samples to validate the onset of occupational exposure to DU.

*Perkin Elmer Corporation, 761 Main Avenue, Norwalk, CT 06859–0012.

2. Experiment

We determined the uranium isotopic composition of 12 urine samples from individuals enrolled in the DU follow-up program [1] at the Baltimore Veterans Administration Medical Center using a Perkin Elmer ELAN 6000 ICP-MS (fig. 1 to 4). Table 1 shows the operational parameters of the ICP-MS used in these measurements.

We operated the ELAN 6000 in a continuous aspiration mode using a Gilson peristaltic pump. We used the dual detector and autolens modes of operation with a DU standard to reduce mass bias effects and obtain ratio correction factors for the ELAN software.

The total concentration of uranium in the urine was first determined at AFRRI with a Chemchek Instruments model KPA-11 kinetic phosphorescence analyzer equipped with a Gilson 222 sample changer and 401 dilutor autosampler. (The principles of KPA have been described in detail elsewhere [7,23,30].) The urine samples for the ICP-MS analysis were processed in 20-mL glass scintillation vials. Samples were initially dried in an oven at 120 °C for 24 hr. They were then dry ashed in a muffle furnace at 300 °C for 24 hr, followed by a 450 °C dry-ash step for 4 hr. After dry ashing, samples were wet ashed with 2 mL of concentrated nitric acid and 0.5 mL of 30-percent hydrogen peroxide. The mixture was heated to just below boiling until evaporation was complete. The residues were cooled and wet ashed three more times in the same manner. The samples were further dry ashed at 450 °C for 4 hr, and then wet ashed two more times with 2 mL of concentrated nitric acid and 0.5 mL of hydrogen peroxide, as described above. At the end of this process, the samples consisted of a white residue that was dissolved in 1 molar nitric acid for ICP-MS analysis. We determined the isotopic composition of uranium by measuring the $^{235}U/^{238}U$ isotopic ratio. Each sample was measured with six replicates and 400 scans per replicate. The percentages of ^{235}U and ^{238}U were calculated from the $^{235}U/^{238}U$ isotopic ratio.

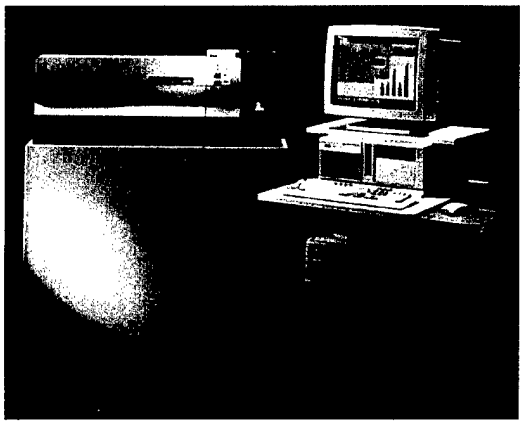

Figure 1. ELAN 6000 inductively coupled plasma mass spectrometer.

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Figure 2. Cutaway showing internal components of ELAN 6000: (A) interface region between plasma torch and mass filter, (B) torch assembly, (C) quadrupole mass filter, (D) ion optics, (E) rf generator, (F) ion detector, (G) turbomolecular pumping system, (H) nebulizer, and (I) spray chamber.

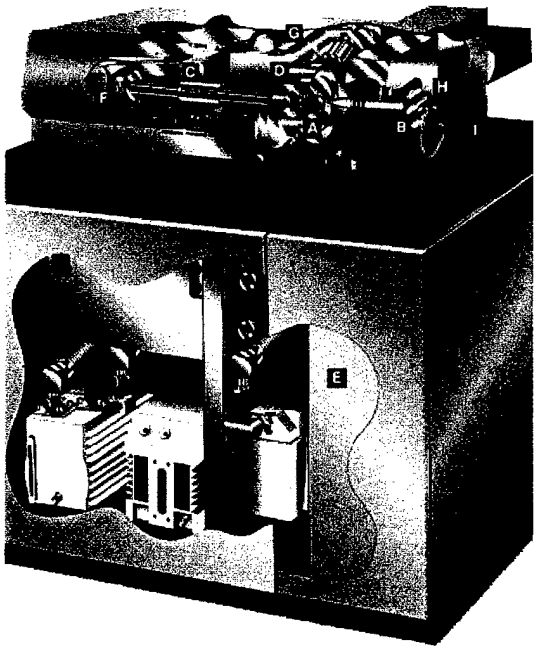


Figure 3. Major hardware components of ELAN 6000 ICP-MS.

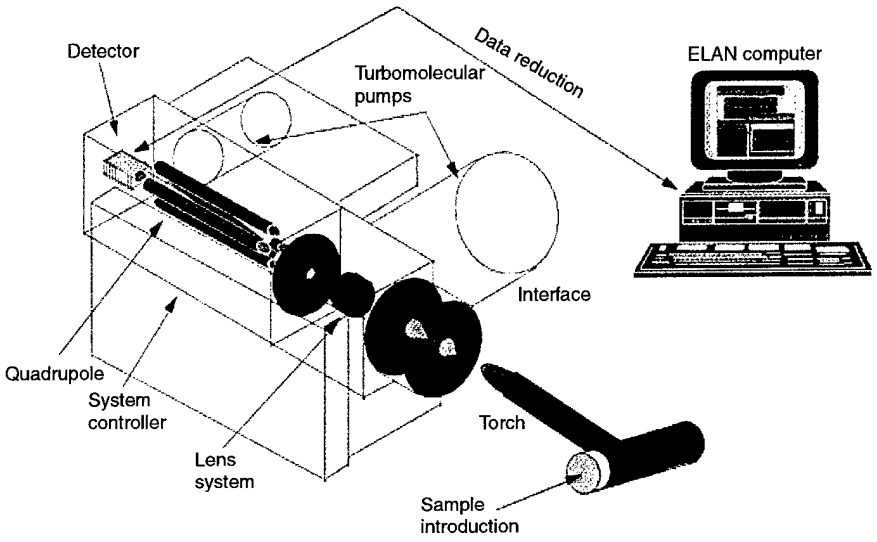


Figure 4. Schematic of components of ELAN 6000 ICP-MS.

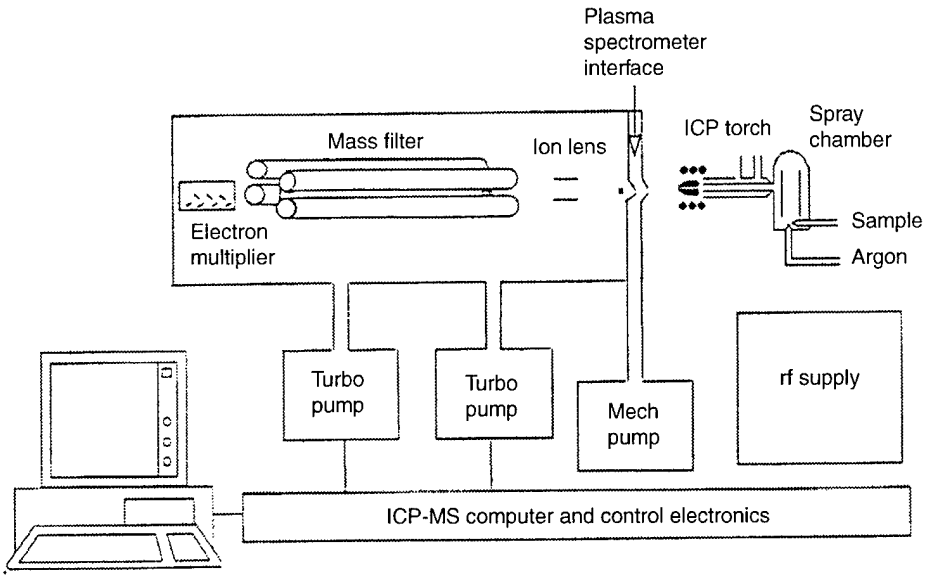


Table 1. Operating conditions used with ELAN 6000 ICP-MS for isotopic ratio measurement of uranium U^{235}/U^{238} in urine.

Instrument operating parameters	Setting
rf power	1000 W
Nebulizer gas flow rate	0.85 L min^{-1}
Auxiliary gas flow rate	1.1 L min^{-1}
Plasma gas flow rate	15 L min^{-1}
Lens setting	8.25 V
Nebulizer type	Cross-flow
Interface cones	Nickel
Mass spectrometer acquisition parameters	
Dwell time	25 ms
Number of sweeps	400
Number of replicates	6
Scan mode	Peak hopping
Multichannel analyzer channels per peak	1

3. Results and Discussion

We can calculate the IDL for uranium by ICP-MS based on 3.29 times the standard deviation of the blank [23,31]. Using this criterion, we determined that the IDL was less than 0.1 ng L^{-1}, a value that is in agreement with Denoyer [32] and Karpas et al [27] and somewhat better than the IDL reported earlier for an ELAN 250 system by Boomer and Powell [15]. Given that the IDL was approximately 0.1 ng L^{-1} and the ^{235}U composition is 0.72 percent for natural uranium and ~0.2 percent for the DU, the $^{235}U/^{238}U$ isotopic ratio measurement limits were 14 and 50 ng L^{-1}, respectively.

A total of 12 urine samples from individuals enrolled in the DU program at the Baltimore Veterans Affairs Medical Center were ashed, dissolved in 1 molar nitric acid, and measured by KPA and ICP-MS (see table 2). For the KPA analyses, we monitored I-methylglycocyamidine, commonly called creatinine, as an internal standard to assess urine dilution.

Of the 12 urine samples measured by KPA, all but samples 7, 10, and 11 had levels of uranium above the normal reported range [27] of 6 to 30 ng L^{-1}. We determined the percentage of ^{235}U from the $^{235}U/^{238}U$ ion intensity ratio measured by ICP-MS (table 2, fig. 5). To calculate the amount of uranium present in the urine from a DU source, we assumed the concentration of ^{235}U in the DU to be 0.2 percent. Table 2 and figure 6 show the calculated percentage of DU in the 12 urine samples.

Of the 12 urine samples, 8 were determined to contain DU, 3 were void of DU, and 1 was below the IDL. Of the eight samples containing DU, six contained >95 percent DU, one contained 90.1 ± 1.6 percent DU, and one contained 74.1 ± 3.8 percent DU.

The six DU samples containing >95 percent DU are not calculated to be 100 percent because the defined range of DU is between 0.20 and 0.25 percent ^{235}U, and we assumed that the ^{235}U content of DU was exactly 0.20 percent. However, the six samples may actually contain 100 percent DU, since the ^{235}U concentration was 0.21 to 0.22 percent, which is within the defined range (0.20 to 0.25 percent). The uranium concentration in these six samples is also elevated significantly relative to normal reported levels of 6 to 30 ng L^{-1} (table 2).

Samples 1, 7, and 10 were void of DU within experimental error and contained only natural background uranium. Samples 7 and 10 were determined to be in the range of normal uranium levels because they contained less than 50 ng L^{-1}. The uranium concentration in sample 1 is above the normal levels reported by Karpas et al [27]. However, even the results for this sample fall within the normal range if we consider the work of Dang et al [33] and Beyer and coworkers [34], where the normal

distribution covered the range from 3 to 310 ng L^{-1}. The uranium concentration in sample 11 was below the IDL. The ^{235}U counts per second (cps) were less than 3.29 times the background intensity for this sample. The high background level yielded a higher count rate at mass 235, which resulted in an apparent enriched ^{235}U percentage.

Table 2. Raw uranium data from urine samples measured by ICP-MS.

Sample ID	^{235}U (cps)[a]	^{238}U (cps)	Ratio $^{235}U/^{238}U$	RSD[b] (%)	^{235}U (%)	DU (%)	U[c] (ng L^{-1})
1	54.7	7,596	0.00734	2.81	0.73±0.020	–1.9±4.0	110
2	374	175,432	0.00215	0.90	0.21±0.002	98.1±0.4	1,400
3	4841	2,178,768	0.00224	0.79	0.22±0.002	96.2±0.4	36,000
4	1588	771,706	0.00208	0.70	0.21±0.001	98.1±0.3	9,000
5	623	300,438	0.00209	0.92	0.21±0.002	98.1±0.4	17,000
6	1523	751,209	0.00205	0.69	0.20±0.001	100.0±0.7	45,000
7	21.2	2,746	0.00777	11.00	0.77±0.085	–9.6±16.3	—[d]
8	75.4	22,634	0.00336	5.87	0.33±0.020	75.0±3.8	150
9	71.4	28,549	0.00252	3.30	0.25±0.008	90.4±1.6	280
10	15.8	2,280	0.00704	14.1	0.70±0.099	3.8±19.0	—[d]
11	6.4	623	0.01029	8.06	1.01±0.081	–55.8±15.7	—[d]
12	1113	544,168	0.00206	1.01	0.21±0.002	98.1±0.4	9,200

[a] Counts per second.
[b] Relative standard deviation.
[c] Uranium concentration measured in urine specimens by KPA. Units are defined as nanograms uranium per liter of urine.
[d] Below detection limit (50 ng L^{-1}) of KPA method.

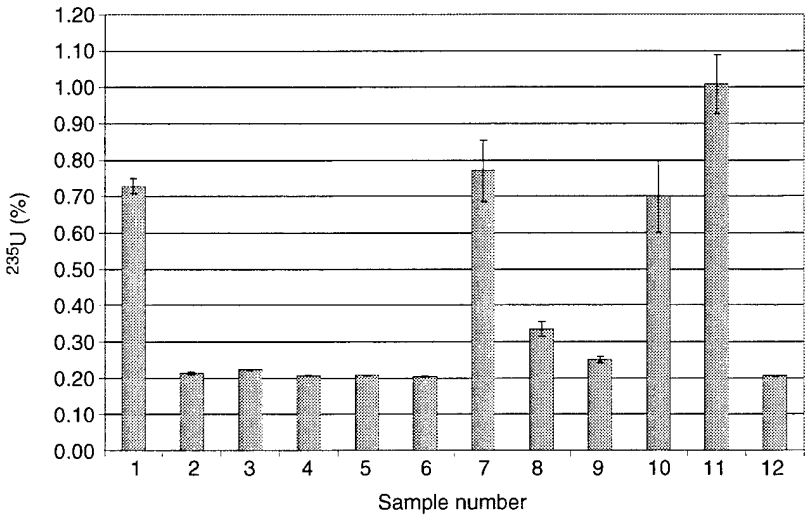
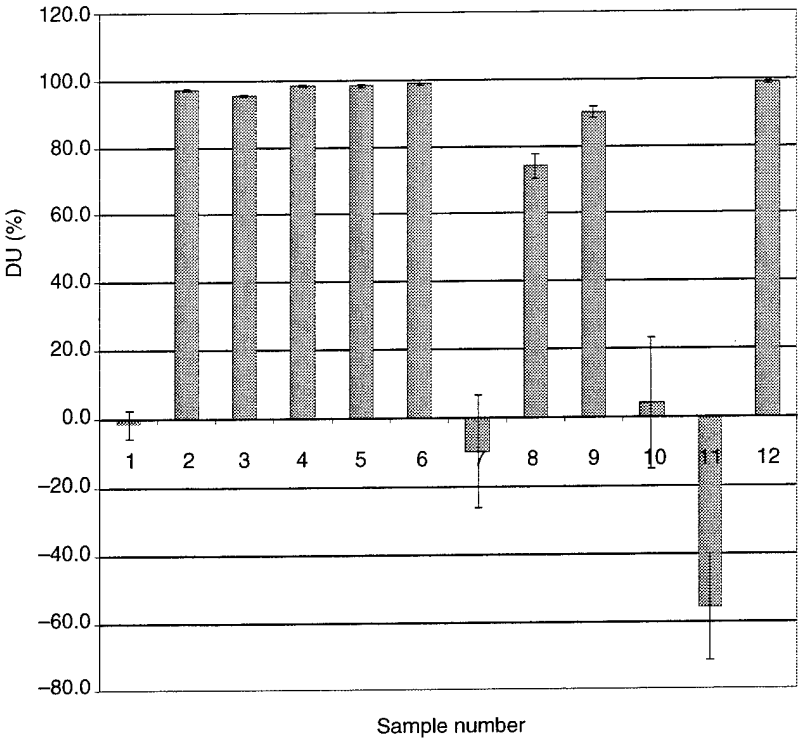


Figure 5. Percentage of ^{235}U in 12 urine samples measured by ICP-MS. Percentages were calculated from measured ^{235}U and ^{238}U intensities.

Figure 6. Percentage DU calculated from percentage of ^{235}U present in urine samples.



4. Conclusions

The urine of individuals not exposed to DU contains natural background uranium, which is 0.72 percent abundant in ^{235}U. Exposure to DU causes the percentage of ^{235}U to decrease to a range between 0.72 and 0.2 wt.% and the total uranium concentration to increase above normal background levels.

The goal of this study was to develop an assay to determine the isotopic composition of uranium in urine samples by ICP-MS, so that we can rapidly and effectively determine exposure of soldiers to DU. Since the detection limit of the instrumentation for isotopic ratio measurements for these kinds of samples (14 ng L^{-1}) is within the range of uranium concentrations in the urine of the typical nonexposed individual (i.e., 6 to 30 ng L^{-1}), the assay can accurately determine if an individual has been exposed to DU when uranium levels in the urine increase above baseline levels. This assay is being used to help determine whether Gulf War veterans with elevated uranium levels have suffered DU exposure.

The entire procedure required 8 days to complete, but over 50 samples can be analyzed per day by ICP-MS operating in the manual sampling mode. The use of an autosampler would increase the throughput at the ICP-MS stage to hundreds of samples per day.

In the past, low levels of DU exposure were difficult to determine because everyone has low levels of natural uranium in their urine. This assay can accurately determine whether the uranium in urine is from DU or natural background uranium. Therefore, this assay can be useful in efforts to resolve toxicological issues relative to DU exposure and health issues associated with the Gulf War.

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13. ABSTRACT *(Maximum 200 words)*
We used a simple method based on inductively coupled plasma mass spectrometry (ICP-MS) to determine the isotopic composition of uranium in urine at levels that indicate occupational exposure to depleted uranium (DU). DU exposure is indicated by a range for ^{235}U between 0.72 and 0.2 percent. Using the ICP-MS isotopic ratio method, we determined the percentage of ^{235}U in a series of urine samples based on the measured intensities of the ^{235}U and ^{238}U ions. In this study, urine samples from 12 individuals suspected of having been exposed to DU were processed by the Armed Forces Radiobiology Research Institute (AFRRI) and then analyzed by ICP-MS at the Army Research Laboratory for the presence of DU. We achieved a lower detection limit of 14 ng L^{-1} in the original urine samples, which is at or near uranium levels in the general population (not occupationally exposed to uranium). We concluded that this approach can be used to determine the percentage of ^{235}U in urine samples characterized by higher than normal uranium levels, and thus whether occupational exposure to DU has occurred.

14. SUBJECT TERMS
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